2000. The Journal of Arachnology 28:128–130

RESEARCH NOTE

EGG SACS OF *PITYOHYPHANTES PHRYGIANUS* ARE NOT AFFECTED BY ACID RAIN

Keywords: Air pollution, reproduction, spider embryos

Acidic precipitation is one of the most important air pollution problems today, causing ecological as well as physiological effects on terrestrial and aquatic animals (Newman et al. 1992). However, the effects on terrestrial arthropods are poorly known. In their review of pollution and insects, Heliövaara & Väisänen (1993) found only three studies focussed on the direct effects of acid rain on terrestrial arthropods. In one of the studies, the growth rate of juvenile spiders exposed to simulated acid rain was examined (Gunnarsson & Johnsson 1989). However, earlier stages during development may be exposed to acid rain as well. Spiders deposit their eggs within an egg sac. The sac is a shelter for the eggs and made of spider silk, consisting of protein with alanine and serine as two major components (Foelix 1996). Each developing spider egg is protected by the chorion layer (Foelix 1996). This means that in order to damage the embryonic spiders, the acidic water must penetrate not only the silken egg case, but also the chorionic egg shell.

Here I examine the effects of simulated acid rain on egg sacs of the spruce-living (*Picea abies* (L.)) sheetweb spider *Pityohyphantes phrygianus* (C.L. Koch 1836). In south Sweden, it has a biennial life-cycle. The males mature before the females in late spring (Gunnarsson & Johnsson 1990), and mating takes place in May. In late June, the females start reproducing, placing their egg sacs directly on spruce branches. This means that the egg sacs in most areas of south Sweden are exposed to ambient concentrations of air pollutants, including acid rain, for about three weeks until hatching starts.

Adult females were collected from spruce branches at different sites in coniferous forests 20–40 km east of Göteborg in SW Sweden. The females were collected at the end of June, when egg production starts. In the laboratory, the females were placed in 0.5 liter plastic vials with spruce twigs. They were fed with vestigial wing fruit flies (*Drosophila melanogaster*) *ad libitum*. The vials were sprayed with tapwater at regular intervals to maintain the humidity. All experiments were performed at room temperature (21–25 °C) and under natural photoperiod.

The females produced a first egg sac, which was attached to a twig or to the inside wall of the vial. The egg sacs were carefully removed and placed individually in 10 ml plastic vials, which were closed with a cotton ball. Approximately 70% of the females produced a second egg sac. All further treatments of the egg sacs were randomized.

Each egg sac, once a day, was gently sprayed with water of a particular acidity, which formed a cover of small drops on the egg sac and the insides of the vials. The spraying was done in a standardized fashion that was similar in all treatment groups. The control group was sprayed with tapwater of pH 7 and the experimental groups with water of pH 4.0 (simulated acid rain; mean of bulk deposition in south Sweden is pH 4.3, see Balsberg Påhlsson & Bergkvist 1995), and pH 2.2. The solutions were obtained by using a stock solution of tap water for all treatments. Parts of this stock solution were mixed with diluted sulphuric acid. The pH of the solutions was checked at regular intervals and found to remain constant. However, new solutions were prepared once during the experiment. This experiment is referred to as the "main experiment."

The egg sacs were checked in two ways. First, spiderlings that had emerged from the egg sac were recorded. If no spiderlings were

observed, the egg sac was opened 25 days after its deposition. Second, the hatching success was established by counting the numbers of hatched spiderlings and dead/undeveloped eggs. In egg sacs where spiderlings emerged spontaneously, the spraying of water was ceased on the day of emergence and all spiderlings and any remaining eggs were checked after another two days.

In the presentation of data, means are given together with their standard deviations. Non-parametric statistical methods were used since non-normality was observed in hatching data and transformation did not change this. All tests were two-tailed.

To provide a comparsion with experimental results, egg sac production in a natural population 40 km east of Göteborg was recorded in July. The number of eggs was counted and used for comparison with the experimental situation. Egg sacs from the wild were not used in any experiment. A field-collected egg sac contained, on average, 43.2 ± 15.9 eggs (n = 17). However, there was a negative correlation between the collecting date and the number of eggs in the natural population (Spearman rank correlation test; $r_s = -0.589$, $P = 0.019$, n = 17). This suggests that females in the wild produced smaller clutches later in the season, possibly because there are fewer eggs in a second egg sac. It is known from other species that females produce fewer eggs in successive egg sacs (Foelix 1996).

The mean number of eggs in an egg sac in the main experiment was 36.4 ± 12.8 (n = 88). The egg numbers in the first and second egg sac were similar (Wilcoxon matched-pairs signed-ranks test; $z = -0.74$, $P = 0.46$, n = 31), and not correlated (Spearman, $r_s = 0.162$, $P = 0.36$, $n = 31$). Egg production in the laboratory was similar to the natural population (Mann-Whitney U-test; $z = -1.48$, $P = 0.14$, $n_1 = 88$, $n_2 = 17$).

Spiderlings emerged spontaneously from egg sacs sprayed with water of different acidity except for those treated with water of pH 2.2. A comparison of egg sacs with spontaneously emerging spiderlings in the main experiment showed a highly significant difference between the treatments ($\chi^2 = 26.20$, df = 2, $P = 0.001$): spiderlings emerged in 72.7% ($n = 33$) of the egg sacs in the control (pH ≈ 7), 65.5% ($n = 29$) in pH 4.0, and 0% ($n = 17$) in pH 2.2.

In the main experiment, the hatching success of the spiderlings in the first and second egg sac was similar within each treatment (Mann-Whitney U-tests; $0.51 < P < 0.75$). Consequently, first and second egg sacs were pooled in the analyses. Comparisons between the treatments (pHs ≈ 7 (control), 4.0 (simulated acid rain), 2.2) showed that the hatching success differed significantly (Kruskal-Wallis one-way ANOVA; $H = 13.43$, $df = 2$, P = 0.0012). Multiple comparisons at the 5% level (Siegel & Castellan 1988), showed that the mean hatching rate in pH 2.2 (13.7% ± 17.0%, $n = 17$) differed from control (51.8% ± 37.2%, $n = 33$) and from simulated acid rain (43.1% ± 38.3%, $n = 29$), but there was no difference between the two latter treatments. Pooling the treatments of pH ≈ 7 and 4.0 revealed a negative correlation between the number of eggs in each egg sac and the hatching success (Spearman, $r_s = -0.504$, P = 0.0001, $n = 62$). This was, however, not the case in pH 2.2 ($r_s = 0.091$, $P = 0.71$, n = 17).

In an additional, small scale experiment one year after the main experiment, treatments with pH ≈ 7 (control), pH 4.0, pH 3.5 and pH 3.0 solutions were performed as in the main experiment. The reason for doing this additional experiment was to examine the effects of another two acidic solutions (pH 3.5 and 3.0), and test for a possible threshold below pH 4.0. This experiment was analyzed separately since it was performed at room temperature, i.e., there were slightly different conditions between years.

In the additional experiment, approximately similar percentages (67–78%) of egg sacs with emerging spiderlings were observed among the groups (pHs ≈ 7, 4.0, 3.5, and 3.0). The hatching success of spiderlings in the treatments pH ≈ 7 (mean 80.9% ± 36.6%, n = 5), pH 4.0 (75.5% ± 35.2%, $n = 8$), pH 3.5 (83.3% ± 31.9%, $n = 9$), and pH 3.0 (68.8% ± 54.0%, $n = 3$) was similar (Kruskal-Wallis one-way ANOVA; $H = 0.25$, df = 3, $P = 0.97$). There was no correlation between the number of eggs in each egg sac and the hatching success (Spearman, $r_s = 0.161$, $P = 0.43$, $n = 25$).

Obviously developing embryos are rather well protected against acid rain since only egg sacs treated with water of pH 2.2 showed a statistically significant deviation from the con-

trol. Examination of the egg sacs suggested that the outside structure of the sacs was affected at this low pH. The silk formed a dense mass of threads, which were glued together but with minute openings in between, in contrast to the loose structure of threads in the unaffected egg sacs. This had two consequences: (1) the hatched spiderlings could not emerge from the egg sac, possibly because they could not find their way out of walls consisting of threads glued together; (2) the hatching of spiderlings was affected negatively, suggesting that acidic water entered the damaged egg sac and reached the developing embryos.

The correlation between egg numbers on hatching success of spiderlings may be an artifact due to disturbance. Removal of egg sacs from the deposition points may have caused unfavorable position changes of eggs within clutches. It is also possible that the water spraying was insufficient to support all eggs in large clutches with enough moisture. In natural populations, the mean hatching success of spiderlings seems to be >90% (pers. obs.). The experimental hatching success was low even in the control, suggesting that the laboratory conditions affected the results, at least in the main experiment. However, the egg numbers per egg sac in the natural population and in the experiment were similar.

The pH of throughfall water in spruce was slightly higher than bulk deposition in south Sweden, averaging 4.3–4.6 (Balsberg Påhlsson & Bergkvist 1995). Thus, there is no evidence suggesting that acid rain affects the development of embryos within spider egg sacs, unless under extreme conditions. Similar results were obtained for growing juveniles of *P. phrygianus* (Gunnarsson & Johnsson 1989). In the present system, indirect effects of acid rain are more important. For instance, accelerated needle-loss is causing changes in predator-prey interactions, involving spiders and their predators (Gunnarsson 1995, 1996; Sundberg & Gunnarsson 1994).

I thank K. Hellström, J. Johnsson and K. Madsen for laboratory assistance. This study was supported by the National Swedish Environment Protection Board.

LITERATURE CITED

Balsberg Påhlsson, A-M. & B. Bergkvist. 1995. Acid deposition and soil acidification at a southwest facing edge of Norway spruce and European beech in south Sweden. *In* Effects of acid deposition and tropospheric ozone on forest ecosystems in Sweden. (H. Staaf & G. Tyler, eds.). Ecol. Bull., 44:43–53.

Foelix, R. 1996. Biology of Spiders. 2nd ed. Oxford Univ. Press, New York. 330 pp.

Gunnarsson, B. 1995. Arthropods and passerine birds in coniferous forest: The impact of acidification and needle-loss. *In* Effects of acid deposition and tropospheric ozone on forest ecosystems in Sweden. (H. Staaf & G. Tyler, eds.). Ecol. Bull., 44:248–258.

Gunnarsson, B. 1996. Bird predation and vegetation structure affecting spruce-living arthropods in a temperate forest. J. Anim. Ecol., 65:389–397.

Gunnarsson, B. & J. Johnsson. 1989. Effects of simulated acid rain on growth rate in a spruce-living spider. Environ. Pollut., 56:311–317.

Gunnarsson, B. & J. Johnsson. 1990. Protandry and moulting to maturity in the spider *Pityohyphantes phrygianus*. Oikos, 59:205–212.

Heliövaara, K. & R. Väisänen. 1993. Insects and Pollution. CRC Press, Boca Raton. 393 pp.

Newman, J.R., R.K. Schreiber & E. Novakova. 1992. Air pollution effects on terrestrial and aquatic animals. Pp. 177–233, *In* Air Pollution Effects on Biodiversity. (J.R. Barker & D.T. Tingey, eds.). Van Nostrand Reinhold, New York.

Siegel, S. & N.J. Castellan. 1988. Nonparametric Statistics. 2nd ed. McGraw-Hill, New York. 399 pp.

Sundberg, I. & B. Gunnarsson. 1994. Spider abundance in relation to needle density in spruce. J. Arachnol., 22:190–194.

Bengt Gunnarsson: Department of Zoology, Box 463, and Department of Applied Environmental Science, Box 464, Göteborg University, SE 405 30 Göteborg, Sweden

Manuscript received 25 February 1999, revised 10 June 1999.